Combination of Probiotics and Sublingual Immunotherapy in Allergic Rhinitis: A Real-Life Study

Renato Rossi[1,*], Lucilla Rossi[2] and Giorgio Monasterolo[3]

[1]*Vita-Salute San Raffaele University, School of Medicine, Via Olgettina n. 58, 20132 Milano, Italy;* [2]*London, UK;* [3]*Racconigi, Cuneo, Italy*

Abstract: Probiotics are increasingly recognized as capable of modulating immune responses. Some probiotic strains show the potential of inducing a different lymphocyte polarization, promoting the Th1 phenotype and inhibiting, at the same time, the allergy-prone Th2 phenotype. On this basis, we could expect that probiotics may act synergistically to improve the clinical efficacy of sublingual allergen immunotherapy (SLIT).

In this study, 30 patients affected by allergic rhinitis undergoing SLIT, were concomitantly administered a probiotic supplement (n = 14) or not (n = 16), according to their preference.

Nasal symptom score, rescue medication score and 'well-days' were evaluated after 2 and 4 months of treatment.

Patients who were administered SLIT plus probiotics showed a trend toward reduction of the nasal symptoms (-7.1%, p = ns) with a significant reduction of medication score (-32.6, p = 0.02) and an increase of 'well-days' (35.1, p = 0.02).

These preliminary data, from a small study population, suggest that this combined approach with SLIT and probiotics could result in an increased efficacy of the SLIT treatment.

Keywords: Probiotics, allergy, allergic rhinitis, immunotherapy.

INTRODUCTION

Gastrointestinal microflora promotes potentially antiallergenic processes: (1) T-helper-1-type immunity (Th1); [1] (2) generation of transforming growth factor ß (TGF-ß), [2,3] which has an essential role in suppression of T-helper-2-induced allergic inflammation [4] and induction of oral tolerance toward allergens; [5] and (3) IgA production, [6] an essential component of mucosal immune defense. *Lactobacillus rhamnosus*, has proved safe even at an early age and an interesting opportunity in the treatment of allergic inflammation [7,8]. In a study by Kalliomaki *et al*., despite a much lower eczema incidence in the *Lactobacillus*-treated infants compared with placebo groups, [9] there were no effects on respiratory disease at 7 year of age. Many of the studies on probiotics as prevention or treatment of allergic diseases focused on *Lactobacilllus spp*.

Lactobacillus rhamnosus LR05, in a small study group, demonstrated a potential beneficial effect on symptom score and medication use in patients affected by atopic dermatitis; Lactobacillus *acidophilus NCFM* and *L. plantarum LP01* together with *L. paracasei* LPC00 demonstrated a similar effect in patients affected by seasonal and perennial allergic rhinitis, respectively [10].

Another study with *L. reuteri* ATCC 55730 (1 × 10(8) CFU) during the last month of gestation and through the first year of life showed no effect on the prevalence of respiratory allergies; [11] it has to be noted, that in this case a low amount of viable cells was administered. However, probiotic bacteria, which affect the host by improving microbial balance, may mediate antiallergic effects by stimulating production TGF-ß, Th1-cytokines and IgG antibodies [4].

From this point of view, *B. lactis* BS01 and *L. rhamnosus* LR05 show an interesting immunomodulation profile: *in vitro* studies demonstrated their capability of stimulating PBMC to produce higher amounts of IFN-γ and IL-12 and regulatory cytokine IL-10, thus promoting the polarization of lymphocytes toward a Th1 phenotype and inhibiting, at the same time, the polarization toward the allergy-prone Th2 phenotype.

On this basis, we could expect that probiotics may act synergistically to improve the clinical efficacy of sublingual allergen immunotherapy. A recent study showed that lung function test improved in patients receiving sublingual allergen-specific immunotherapy (SLIT) together with probiotics [12]. Another study showed that sublingual administration

*Address correspondence to this author at the Vita-Salute San Raffaele University, School of Medicine, Via Olgettina n. 58, 20132 Milano, Italy;
Tel: +3902 91751.549; Fax: +3902 91751; E-mail: immunoway@libero.it

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of *B. bifidum* together with recombinant Bet v 1 enhanced tolerance induction in BALB/c mice sensitized to birch pollen, with a downregulation of both airway hyperresponsiveness, lung inflammation and Bet v 1-specific Th2 responses [13]. A recent study with a mouse model of allergic asthma showed that oral administration of *L. gasseri* attenuated allergen-induced airway inflammation and induced a reduction in IL 17-mediated immune response [14].

The purpose of this study was to investigate the safety of co-treatment of SLIT and probiotics and their possible positive effects on allergic symptoms.

PATIENTS AND METHODS

The present real life study involved 30 allergic subjects with allergic rhinoconjunctivitis and/or asthma. Demographic and clinical (age mean and range, male to female ratio, diagnosis) of the study population are showed in Table **1**.

All the enrolled patients underwent an allergologic workout, comprehensive of component-resolved tests, and a three visits study protocol. Sensitizations and serological characteristics of the study group are depicted in Table **2**.

Study Protocol

At visit I, a comprehensive medical/medication history was taken, clinical examination and skin prick test were performed. In the case a clinical diagnosis of allergic rhinoconjunctivitis (and eventually asthma) was confirmed, pharmacological treatment was prescribed, according to relevant guidelines [15]. Add-on optional therapeutic options (SLIT and probiotics supplementation) were discussed with the patient; if indicated, these were prescribed according to patients' preferences. Patients willing to start a course of SLIT therapy were enrolled in the study population, and assigned to the corresponding treatment group (SLIT

Table 1: Study Population

	Mean age (range)	M/F	Diagnosis	
Group 1 (SLIT)	34.9 (20-63) years	10/6	RC, n=11	RCA, n=5
Group 2 (P+SLIT)	31.7 (19-70) years	6/8	RC, n=10	RCA, n=4

R, rhinitis; C, conjunctivitis; A, asthma; M, male; F, female.

Table 2: Serological Characterization of the Study Population

Group 1 (SLIT)			Group 2 (P+SLIT)		
N	**PPT**	**Total IgE**	**N**	**PPT**	**Total IgE**
1	m, c	47	1	m, mo	144
2	g, m	121	2	b, g, o	177
3	g, b, o	266	3	b, o	115
4	m, mo	176	4	g, m	224
5	b, o	22	5	g	287
6	b, w	155	6	g, w	164
7	m, c	121	7	g, o, w	327
8	g, o, w	412	8	b, o	128
9	m, c	89	9	w	435
10	o, w	66	10	o	36
11	mo	43	11	g	217
12	b, o	185	12	m, c, g	245
13	b, g	344	13	mo	32
14	m, c	321	14	b,o,g	367
15	m, c, g, w, b	612			
16	g, w	96			

Positive prick test, PPT: g, grass; m, mites; b, birch; w, weeds; o, olive tree; c, cat; mo, moulds. Total IgE are expressed in kU/l.

Table 3: Score for Allergic Rhinitis Questionnaire (SFAR) [15]

Q1. In the past 12 months (or since last visit), have you had a problem apart from cold or flu with (please tick appropriate cases(s)):

- Sneezing No Yes
- Runny nose No Yes
- Blocked nose No Yes
- Asthma No Yes
- Cough No Yes

Q1b. If YES (at least one nose problem): in the past 12 months (or since last visit), has this nose problem been accompanied by itchy-watery eyes?

No Yes

Q2. In which of the past 12 months (or in which season) did this nose problem occur?

Jan Feb Mar Apr May June July Aug Sept Oct Nov Dec

(or alternatively)

Winter Spring Summer Autumn

Q3. What trigger factors provoke or increase your nose problem?

- House dust No Yes
- House dust mites No Yes
- Pollens No Yes
- Animals No Yes (please specify:)
- Others No Yes (please specify:)

Q4. Do you think to be allergic?

No Yes

Q5. Have you already been tested for allergy (SPT, IgE)?

No Yes

Q5b. If YES: What was the result?

Positive Negative

Q6. Has a doctor already diagnosed that you suffer/suffered from asthma, eczema or allergic rhinitis?

No Yes

Q7. Is any member of your family suffering from asthma, eczema or allergic rhinitis?

No Yes

Q7b. If YES: Who and what disease? (please tick appropriate cases(s)):

Father	Asthma	Eczema	Allergic rhinitis
Mother	Asthma	Eczema	Allergic rhinitis
Siblings	Asthma	Eczema	Allergic rhinitis

or SLIT plus probiotics) according to their preference regarding the administration of a concomitant probiotics supplementation.

Exclusion criteria were previous treatment with SCIT or SLIT, and permanent treatment with intranasal or systemic corticosteroids within the last 4 weeks prior to the start of the study.

Enrolled patients were asked to keep a daily record of their symptoms relating to eyes, nose and/or lung and of any concomitant medication (Table **3**); the standardized "score for allergic rhinitis questionnaire" (SFAR) was used for this purpose [15].

At visits II (after 2 months) and III (4 months), a follow-up evaluation was performed and clinical and medication scores were collected.

In Vitro Tests

IgE-specific antibodies for recombinant and purified allergenic molecules (rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl 6, rPhl p 7, rPhl 11, rPhl 12, Der p 1, Der p 2, Bet v 1, Par j 2, Ole e 1, Art v 1, Alt a 1) were evaluated using the immuno-enzymatic CAP system (Thermo Fisher Scientific, Uppsala, Sweden) following the manufacturer's instructions. The results were expressed in classes of positive results from 0 to 6,

where class 0 corresponds to <0.1 kUA/l; class 1, 0.1–0.7 kUA/l; class 2, 0.7–3.5 kUA/l; class 3, 3.5–17.5 kUA/l; class 4, 17.5–50 kUA/l; class 5, 50–100 kUA/l; and class 6, >100 kUA/l.

Treatment Protocol

The patients were enrolled and assigned to one of two groups on the basis of selected treatment:

- Group 1, SLIT treated individuals (SLIT group, n = 16)
- Group 2, SLIT and probiotic treated individuals (P+SLIT group, n = 14)

Both treatment groups were administered Allergen specific immunotherapy with a sublingual allergen extract (Oralvac Plus®, Allergy Therapeutics LTD, Worthing, UK) according to the manufacturer's suggested dosing schedule. Group 2 also received an industrial combination of *Lactobacillus rhamnosus* LR05 (≥ 10^9 UFC/sachet), *Bifidobacterium lactis* BS01 (≥ 10^9 UFC/sachet) and FOS (Actilight 950P 2.5 g/sachet) (Kallergen Th®; Allergy Therapeutics, Milan, Italy).

Group 2 began probiotics administration 14 days before the first ITS sublingual administration (day 0) and continued for 4 months thereafter, even in this case according to the manufacturer's suggested dosing schedule (rush protocol).

The allergen content of Oralvac Plus® for the specific allergens, as declared by the manufacturer, was as follows: Der p 1, 13.8 µg/ml; Phl p 5, 8.7 µg/ml; Bet v 1, 36 µg/ml; Art v 1, 38.3 µg/ml; Par j 1, 14.8 µg/ml; Alt a 1, 0.7 µg/ml. Proteomic identification and standardization of major allergens was established by High Performing Standardization (HHPS), a company proprietary method which is based on a double mass spectrometry.

Of the 30 patients receiving SLIT, 12 were treated with mite extract (Der p 1 and/or Der p 2 sensitized), 6 with grass extract (Phl p 1 and/or Phl p 5 sensitized), 5 with birch extract (Bet v 1 sensitized), 3 with olive tree extract (Ole e 1 sensitized), 2 with pellitory extract (Par j 2 sensitized), 1 with mugwort extract (Art v 1 sensitized), 1 with *Alternaria alternata* extract (Alt a 1 sensitized), respectively.

In patients showing a poly-sensitization towards respiratory allergens, choice of the SLIT allergen was performed taking into account IgE-sensitization to allergen specific (not cross-reactive) molecules (e.g. Bet v 1 for birch) and peak period of symptoms.

Statistics

For rhino conjunctivitis medication score a Wilcoxon rank sum test was used to test for differences. Statistical analysis was performed with MS Excel.

RESULTS

No relevant local or systemic adverse reactions were reported in any of the three groups of patients. This confirms the safety profile of Kallergen Th® and Oralvac Plus®, either when combined. The average symptom and medication scores were lower for subjects treated with combination of probiotics plus SLIT (Table **4** and Figure **1**) respect to patients treated with either one alone. A mean reduction of 7.1% in rhino conjunctivitis symptom score, without reaching statistical significance. Notably, a 32.6% reduction in rhino conjunctivitis medication score was found (SLIT plus probiotics vs SLIT alone, p = 0.02). In addition, the mean percentage of 'well days' in the pollen season (spring for pollen and autumn for mite

Table 4: Average Medication Scores, Symptom Scores and Percentage of Well Days at Visit II Compared to Baseline

Treatment group	SLIT n=16	P+SLIT n=14	Difference (%)*	*P* value
Rhinoconjunctivitis symptom score				
Nasal symptoms reduction	66.7%	71.4%	7.1	ns
Rhinoconjunctivitis medication score				
Daily medication score	46	31	–32.6	0.02
Well days				
Well days	36.2%	48.9%	35.1	0.02

*Difference (%) of symptoms reduction was calculated as follows:
100 x [P+SLIT – SLIT] / SLIT

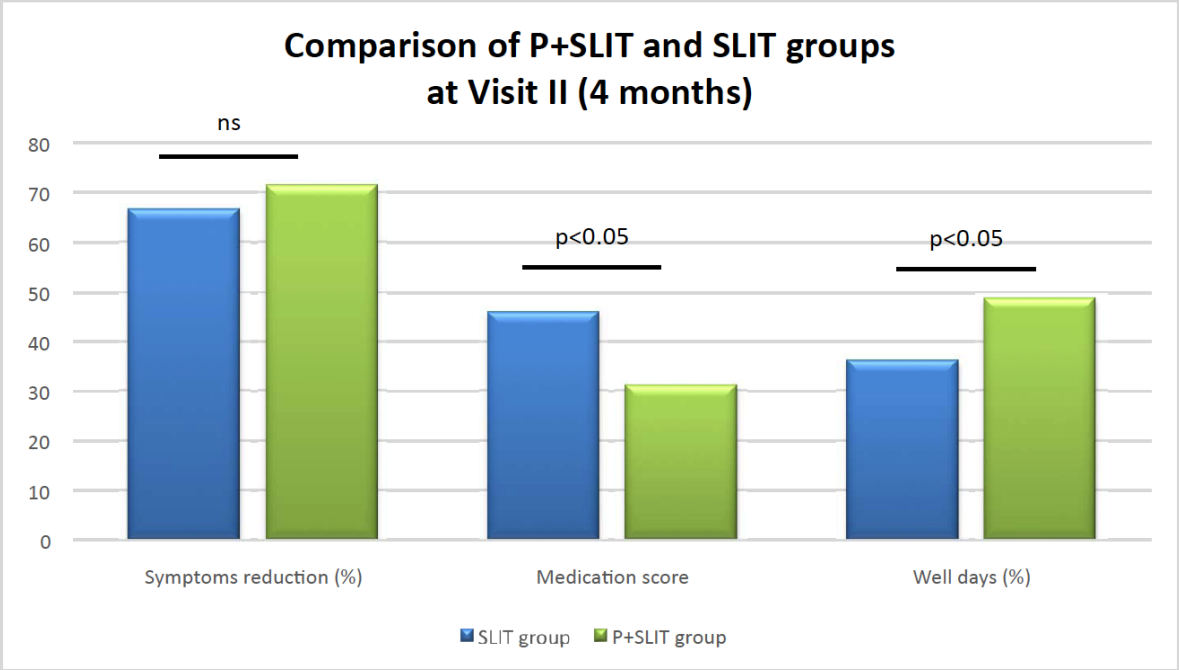

Figure 1: Symptom scores, medication scores and percentage of well days at Visit II (SLIT group vs P+SLIT group).

or mould-allergic patients) was 35.1% higher in the P+SLIT group, respect to the SLIT group (p = 0.02).

DISCUSSION

SLIT has demonstrated to have a role in the management of allergic rhinitis and rhinoconjunctivitis for many years now: it is efficacious on nasal and ocular symptoms and also has a disease-modifying effect [16].

It was previously shown that the intestinal microbial community of non-allergic subjects was found to be more colonized by lactic acid bacteria (LAB), (i.e. *Lactobacillus*) and other bacteria belonging to *Bifidobacterium* genus, demonstrating that the enhanced presence of these bacteria in the gastrointestinal tract seems to correlate with protection against allergic diseases [14,17]. Another study revealed that co-incubation of peripheral blood mononuclear cells from allergic subjects with a variety of LAB strains inhibited allergen-stimulated Th2-cytokine release and increases the Th1-cytokine response [18]. Charng *et al*. demonstrated that mice intraperitoneally sensitized with *Dermatophagoides pteronyssinus* group 5 allergen (Der p 5) and orally treated with recombinant LAB containing a plasmid-encoded Der p 5 gene had reduction in the synthesis of Der p 5-specific IgE [20]. The reported data suggest that many probiotics may share common properties but their effect may be strain-specific [10].

In this study, both groups (SLIT and P+SLIT) showed a marked improvement of total nasal symptom score during the study period, with the P+SLIT group showing a trend to an even better improvement (71.4% vs 66.7%, not reaching statistical significance) respect to the SLIT group.

Increasing the size of the study population could possibly make this difference statistically significant. Interestingly, in the same period, patients belonging to the P+SLIT group had a significant reduction of the medication score (-32.6% vs SLIT group) and a greater number of 'well days' (+35.1% vs SLIT group).

In the P+SLIT group, a symbiotic formulation (P+SLIT group) of *Bifidobacterium lactis* BS01, *Lactobacillus rhamnosus* LR05 and FOS was administered together with the SLIT. These probiotic strains were selected on the basis of their interesting *in vitro* immunomodulation profile: capability of stimulating PBMC to produce higher amounts of IFN-γ and IL-12 and regulatory cytokine IL-10, thus promoting the polarization of lymphocytes toward a Th1 phenotype and inhibiting, at the same time, the polarization toward the allergy-prone Th2 phenotype [21].

Other Authors reported similar experiences with different probiotic strains which detain similar or even more pronounced characteristics in this regard. Ouwehand *et al.* [22] used a mixture of *Lactobacillus acidophilus* NCFMTM (ATCC 700396) and *Bifidobacterium lactis* Bl-04 (ATCC SD5219) in a

double-blind placebo-controlled study for 4 months, starting prior to the onset of the birch pollen season. In this study, the administration of probiotics resulted efficacious in preventing the pollen-induced infiltration of eosinophils into the nasal mucosa, and found a trend toward a reduction of nasal symptoms.

Also Manzotti *et al*. [23] showed the effect of *Lactobacillus acidophilus* NCFM/*Bifidobacterium lactis* BL-04 / fructooligosaccharide preparations in the routine clinical management of subjects with seasonal allergic rhinitis over a period of 4 months. After the treatment with two multi-strain symbiotic preparations a significant reduction of total nasal symptoms and a shift toward a lower level of the ARIA classification of rhinitis were observed. In addition, a decreased consumption of orally-administered corticosteroids and antihistamines drugs was found.

In conclusion, combined self-administration of Oralvac Plus® SLIT together with the symbiotic KallergenTh® was safe and well-tolerated, as no relevant adverse effects were reported. These preliminary data, from a small study population, suggest that this combined approach with SLIT and probiotics could result in an increased efficacy of the SLIT treatment. Further studies are needed to confirm and expand these results, to identify the most suitable probiotic strains for this purpose and to clarify the underlying mechanisms of action.

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